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Investigation of binding mode of isoamyl derivative of thiosalicylic acid and human serum albumin

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pharmaceuticals MDPI

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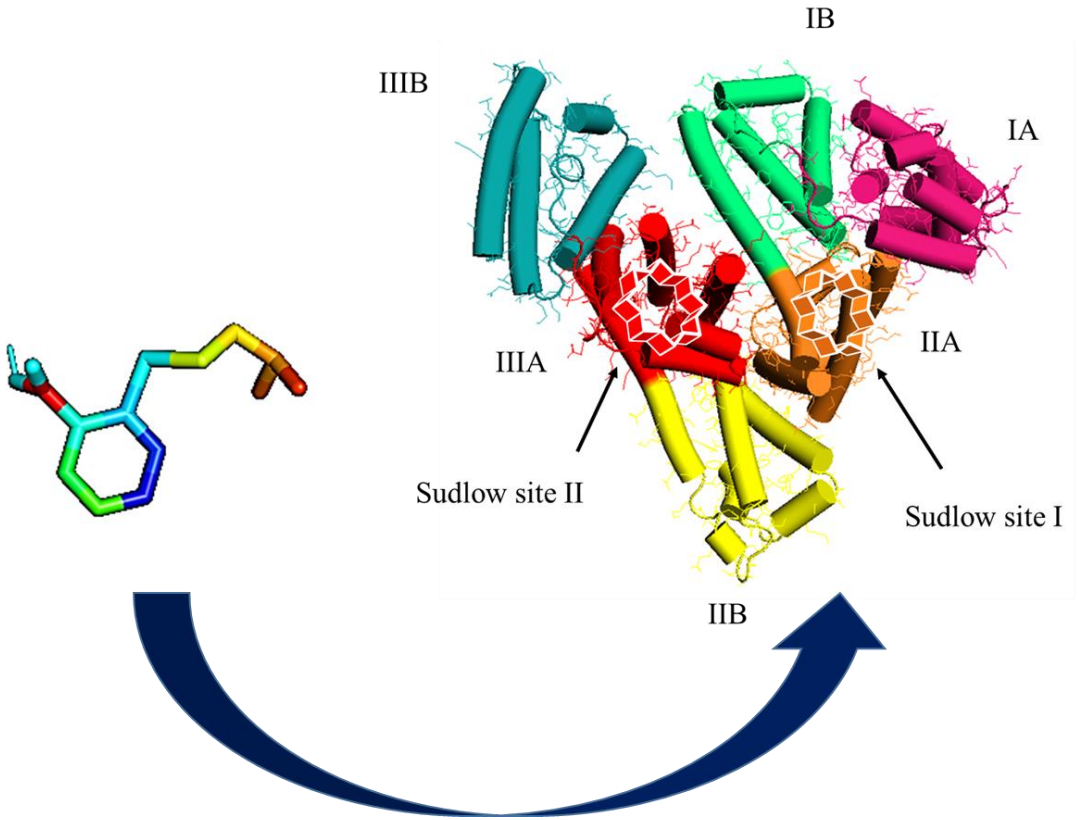
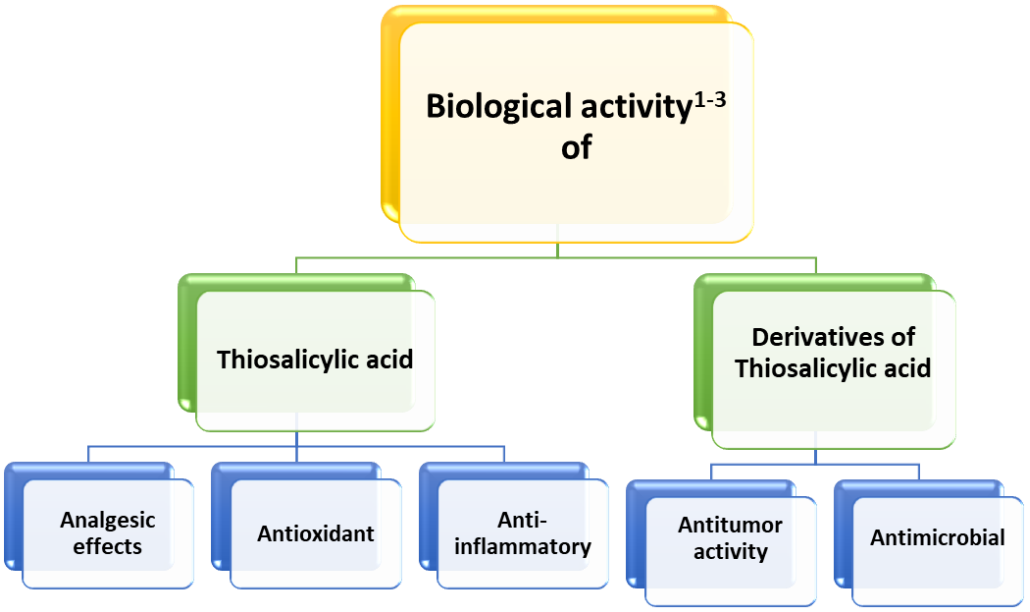


Abstract:

Thiosalicylic acid is known for its diverse pharmacological properties and its frequent use in various synthetic conversions. The unique structure of thiosalicylic acid permits the incorporation of diverse S-alkyl derivatives, resulting in favorable chemical characteristics for numerous applications. Therefore, affinity for one of the binding sites of human serum albumin (HSA) of isoamyl derivative of thiosalicylic acid (ligand, L), were examined. Binding mode of compound L was determined using fluorescence spectroscopy. Warfarin was used as a marker for Sudlow's Site I (subdomain IIA), while ibuprofen was used as a marker for Sudlow's Site II (subdomain IIIA). Obtained values of *K*a suggested that investigated compound bind to HSA. Results of site marker competitive experiments showed that the tested L bind to HSA in domain IIA (Site I). The presented results will help to improve the research of the mechanisam of the interaction between transport proteins and similar compounds.

Keywords: Human serum albumin; S-Isoalkyl derivatives; Spectroscopic Measurements; Thiosalicylic acid

Introduction



- Thiosalicylate component of thimerosal can inhibit the release of vascular endothelial growth factor, thus reducing the toxicity [4,5].
- *In vitro* studies of the antitumor activity indicate that S-alkyl derivatives of thiosalicylic acid exhibit moderate and dose-dependent cytotoxic effects on human colon and lung carcinoma cells [6].

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Introduction

Human serum albumin (HSA)

- Is a central plasma protein, plays a crucial role in:

- The plasma osmotic pressure regulation
- The distribution of fluids in the body
- It's high binding capacity is a very significant factor in the pharmacokinetics of many drugs

- Crystal structure of HSA revealed:

- three domains (I, II, III)
- two subdomains (A and B) within each of domain [7]
- two main binding sites in subdomains IIA and IIIA

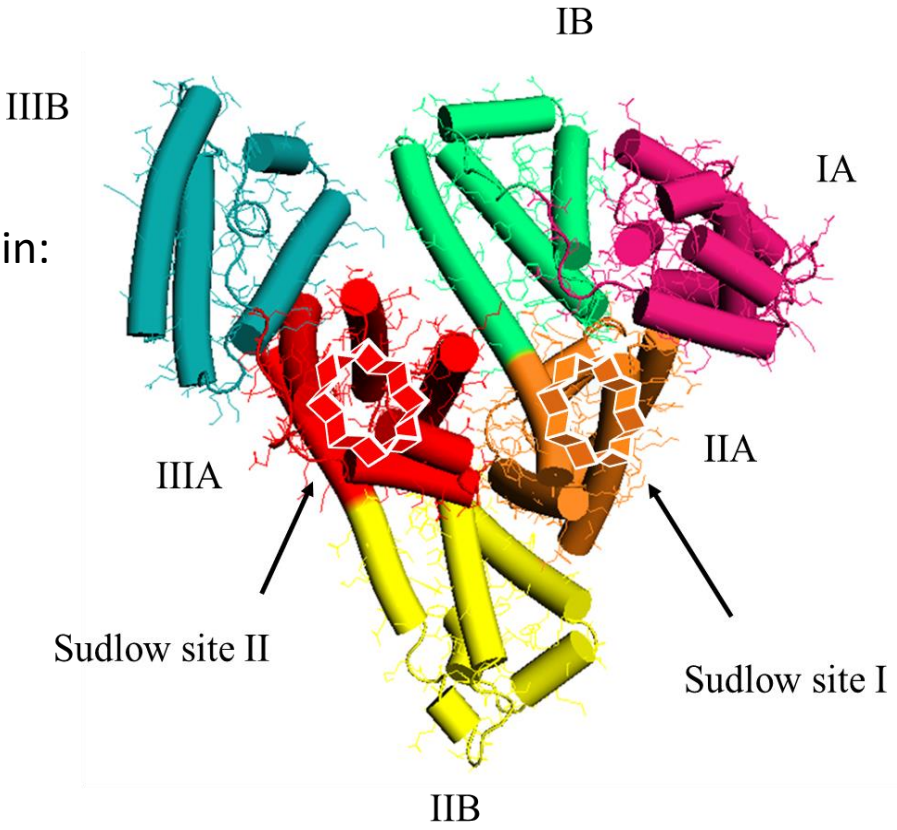

Figure 1. Crystal structure of HSA

References:
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Results and discussion

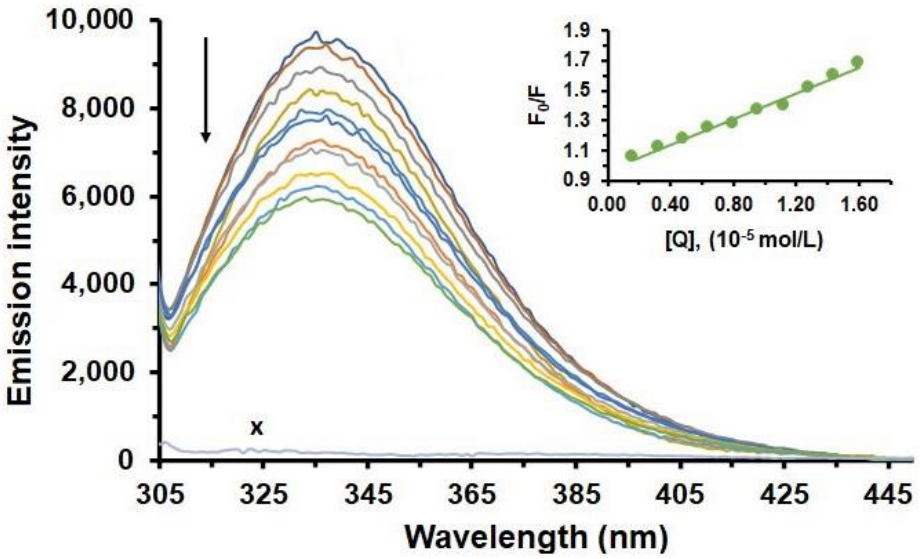

Figure 2. Emission spectra of HSA in the presence of various concentrations of S-isoamyl derivative of thiosalicylic acid (L) (T = 296 K, pH = 7.4). [HSA] = 1.6 µM; [ligand] = 0 - 16 µM. x represents a 16 µM ligand only. The inset: plot of F_0/F vs. [ligand].

Figure 2 displays the fluorescence spectra of HSA and L.

The increasing concentration of L led to decreasing fluorescence intensity, while the shape of the peaks remained nearly consistent over time.

The inset of Figure 2 presents a linear Stern-Volmer plot which indicate that static or dynamic [8] mechanism of quenching occurs. Analyze the *K*q constant (greater than 2.0 x 10^{10} M^{-1} s^{-1}), it can be concluded that the quenching mechanism is probably a static quenching process.

References:
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Results and discussion

Table 1

Binding constants (K_a), and number of binding sites (n) for the interaction of ligand (**L**) with the **HSA-marker** system.

Ligand	K_a (M^{-1})	n	R^{2a}	K_a (M^{-1})	n	R^{2a}
	HSA-IP system			**HSA-WF** system		
L	4.84×10^5	1.17	0.9983	5.30×10^3	0.78	0.9665

[a]R is the correlation coefficient

In the site marker competitive experiment, site markers warfarine and ibuprofen (WF, IP) were used. In all experiments, HSA and WF or IP held in equimolar concentrations (1.6×10^{-6} mol/dm^3). The increasing concentration of L was gradually added to solution of HSA-WF (IP). By examining the fluorescence spectrum before and after introducing WF and IP to the HSA-L system, we can determine that the presence of WF has a significant impact on the binary system's *K*a (3.24×10^5). As a result, it can be assumed that L binds to site I on the HSA.

Results and discussion

In this study, we used spectroscopic methods to identify binding site of tested compound. Then, we predicted the binding mode using AutoDock Vina [9] software through *in silico* analysis. The validation of the molecular docking was confirmed by re-docking the co-crystallized ligand, whereby the calculated RMSD value was 0.513 Å (Figure 3).

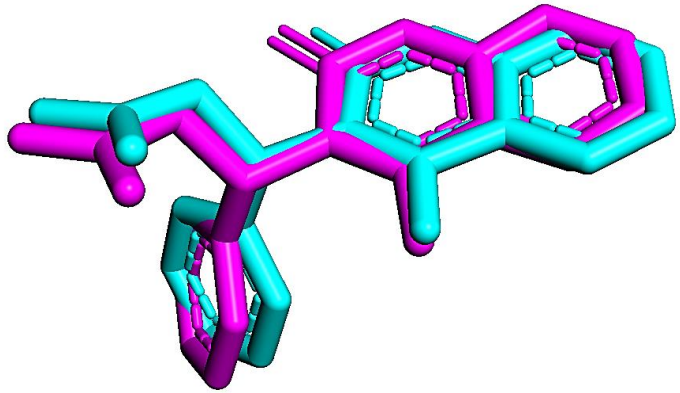

Figure 3. Overlapping of WF native conformation (colored pink) and its re-docked binding pose (colored cyan).

Reference:
9. O. Trott, A.J. Olson, Journal of computational chemistry, 31(2) (2010) 455-461.

Results and discussion

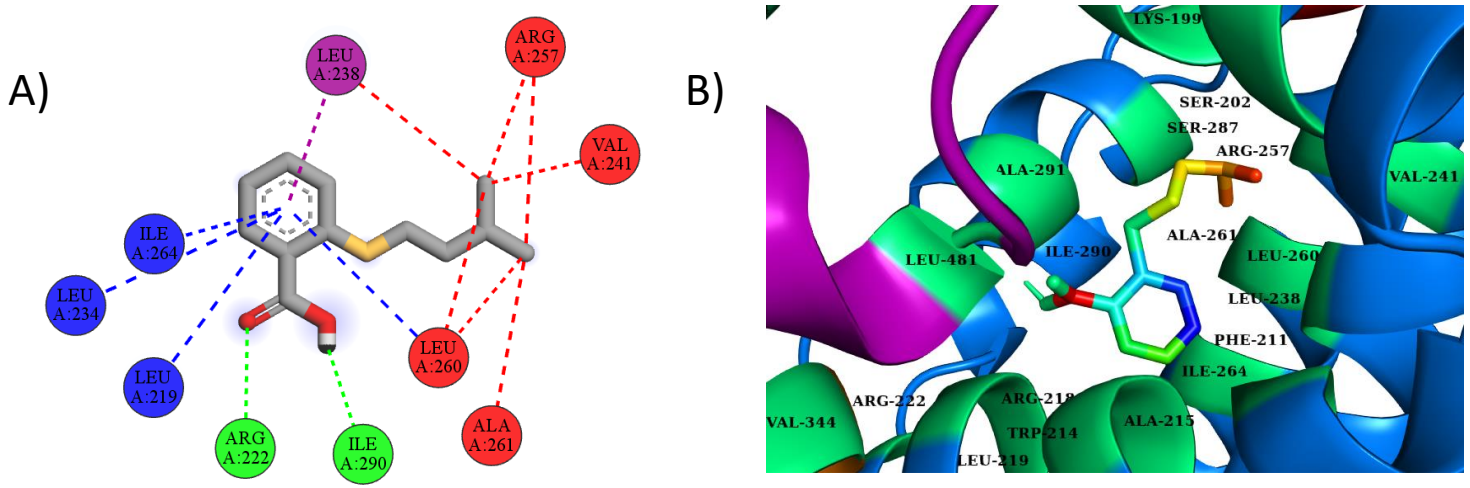

Figure 4. A)Two-dimensional representation of the interaction between L and HSA, B) Three-dimensional display of the residues involved in binding interaction formation between L3 and HSA.

The results of molecular docking confirmed the binding site of tested compound determined by spectroscopic studies. This binding site represents a cavity within a subdomain IIA, which is composed mostly of non-polar amino acid residues. Binding affinity of tested compound was determined based on three parameters, category, number of key interactions, and binding energy. In addition to the hydrophobic interactions, L also forms 2 conventional hydrogen bonds with key amino acid residues, which is also observed when WF is fitted into the binding pocket of albumin.

Conclusions

Interaction of S-isoamyl derivative of thiosalicylic acid and human serum albumin was investigated using fluorescence spectroscopy and molecular docking simulations. Accordingly, we concluded:

1. That the fluorescence quenching mechanism of the S-isoamyl derivative of thiosalicylic acid and HSA was static.

2. The S-isoamyl derivative of thiosalicylic acid bind to the Sudlow site I of HSA, through hydrogen bonds and hydrophobic interactions.

3. The stability of the complex L-HSA is mainly caused by formation of strong non-covalent hydrogen bonds that are maintained over time.

Acknowledgments

ИНСТИТУТ ЗА ИНФОРМАЦИОНЕ ТЕХНОЛОГИЈЕ КРАГУЈЕВАЦ
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